Cancer Immunology Research

High-Dimensional Profiling of Tumor-Specific Immune Responses: Asking T Cells about What They "See" in Cancer

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Abstract

The foundations of basic T-cell immunology and an understanding of the roles for T cells in controlling cancer have led to the remarkable yet inconsistent success of cancer immunotherapy. Because of these advances in cancer treatment, the need is urgent for biomarkers that can predict the efficacy of these treatments and for new therapeutic strategies for cases where currently available approaches are ineffective. Although our ability to profile heterogeneous cell populations in human blood or tissue samples has vastly improved in the past decade, identifying the cell subsets relevant to diseases, and to cancer particularly, remains a challenge. Given strong evidence for the implication of T cells specific for tumor-expressed antigens in various forms of effective immunotherapy, here, we focus on the utility, challenges, and techniques for the identification and profiling of these important cells. We review recent techniques that allow identifying and profiling of tumor-specific T cells. As these methods improve, we can expect more rapid progress in the rational design of novel cancer biomarkers and therapies based on antigen-specific T cells. *Cancer Immunol Res; 6(1); 2–9. ©2018 AACR.*

Introduction

The successes of immunotherapies across disciplines, and especially for cancer, are providing tangible testaments to the importance and validity of the concepts of basic T-cell immunology developed over the past several decades. Fundamental cellular immunology and the molecular basis for T-cell antigen recognition (i.e., how T cells "see" antigens; ref. 1) and T-cell receptor (TCR) signaling (2, 3) have led to the discovery of costimulatory and coinhibitory molecules that modulate T-cell responses, which have proven to be extremely effective targets for modulating immune responses (nicely summarized in ref. 4). In parallel, the development of concepts related to immunosurveillance and immunoediting in cancer has led to a deeper understanding about the roles of antigen-specific T cells in controlling cancer (5) that are leading to novel therapeutic approaches, such as those based on vaccines (6–8). Thus, reductionist systems, including studies with murine model systems and model antigens, have been critical to deciphering the molecular pathways involved in tumor immunology, which have led to these breakthroughs in cancer treatment. However, it is also clear that immune responses in genetically diverse, pathogen-exposed humans are significantly different and more complex than what can be accurately recapitulated in model systems (9). In this setting, which involves heterogeneity of cellular subsets as well as diverse responses observed between individuals, systems level approaches can be particularly effective. These approaches focus on quantitatively probing a biological system (e.g., the immune system) on many axes simultaneously to capture part of its complexity through integrative analysis. The broad profiling of immune cell subsets and other immunologic measures, such as cytokine concentrations and cellular localizations, interactions, and functions, are then used to identify associations that provide novel insights into immunologic mechanisms (10–12). Thus, in contrast to a reductionist approach, systems-level approaches benefit from the heterogeneity seen in the cellular profiles determined for each individual and in terms of the diverse responses observed between individuals. Because the power of these approaches scales with the breadth, depth, and relevance of the immunologic profiles that can be measured, technological advances in the quantification and characterization of additional relevant aspects of immune responses will be critical. Here, we will discuss methods for identifying and profiling the cellular immune response with a focus on antigen-specific T cells in the context of cancer.

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From Targeted to Unbiased Approaches in Immune Biomarker Discovery

The remarkable yet mostly unpredictable success of cancer immunotherapy calls desperately for more accurate methods of predicting the efficacy of these treatments and the identification of alternate means to instill effective immune responses in those who do not respond to current approaches (13). Ongoing success and excitement in the field has also spawned the development of many new approaches aimed at restoring or establishing effective tumor-specific immune responses that are being tested alone or in various combinations. Biomarkers that can quickly assess the utility of an approach are needed so that effective treatments can be started or continued without wasting time on ineffective ones (14).

A large fraction of our current ability to predict cancer patients' prognosis and response to treatments is due to retrospective

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analyses of large cohorts of cancer patients that allowed the identification of correlates of patients' survival. The Union for International Cancer Control's primary tumor, node involvement, and metastasis presence (UICC-TNM) classification, which relies mostly on the assessment of the tumor size and dissemination, has been the gold standard for the prediction of cancer patients' prognosis for decades. Its yet-unmatched predictive power is largely due to a gradual refinement of the classification criteria used by each successive edition (https://books.google.ca/books?id=642GDQAAQBAJ).

A similar trend has been at play in cancer immunology, where this century has witnessed the ground-breaking identification of immune biomarkers in cancer. Using IHC semiquantification, Zhang and colleagues observed that infiltration by $CD3^+$ T cells is associated with longer disease-free survival in ovarian cancer (15). A few years later, Galon and colleagues improved on these findings by using a numerical quantification of $CD3^+$ T cells both in the tumor and its invasive margin, which revealed that this immune biomarker was statistically independent of the TNM classification in colorectal cancer (16). These findings have since been extended to other malignancies, highlighting the association between a high density of $CD8^+$ T cells and favorable outcome in most solid tumors (17). An overall favorable association with prognosis was also observed for B cells, natural killer (NK) cells, dendritic cells, and M1 macrophages (17, 18), whereas M2 macrophages (17, 18) are mostly associated with a poor outcome. The association with macrophages in general and regulatory T cells (Treg) remains unclear (17). In parallel with these gene expression analyses, targeted gene expression studies confirmed the association of a gene module characterized by the expression of IFNγ, with favorable clinical outcomes (15, 17), consistent with the hypothesis that $CD8^+$ T cells were involved in tumor control and associated with slower disease evolution.

Coincidentally, genomics has experienced large technical improvements that popularized whole-genome approaches instead of targeted approaches. The application of DNA microarrays, and then whole-exome mRNA sequencing technologies, to large cohort of cancer patients enabled the unbiased definition of tumor molecular subtypes through the use of clustering techniques, first in breast cancer (19) and in B-cell lymphoma (20). In colorectal cancer, where multiple molecular classification systems have been proposed and unified (21), these unbiased molecular definitions have been associated with prognosis (21, 22), as well as with response to targeted therapies, and could represent diseases originating from distinct precursor cells. Broad pathway analyses first hinted at an association between inflammatory gene signatures and these molecular stratifications (21, 22).

More refined immunologic descriptions of the tumor microenvironment are enabled by algorithms that are specifically designed to leverage the footprints that infiltrating immune cells imprint on the tumor transcriptome, so-called deconvolution methods. These methods, such as CIBERSORT or MCP-counter, usually rely on reference transcriptomic profiles obtained from sorted immune populations (23, 24). These methods legitimize more precise statements about the composition of the tumor microenvironment of each of these subgroups (25, 26) and confirmed previous observations, such as the association between the microsatellite-instable phenotype and tumor microenvironments rich in cytotoxic T cells with high IFNγ expression. New immune subgroups have been characterized with these methods, such as the highly myeloid, angiogenic, fibroblast-rich, poor-prognosis "mesenchymal" subtype in colorectal cancer (25). However, these algorithms, although valuable in supporting more refined immunologic statements emerging from broad genomic analyses (27), are limited to predefined and sorted cell populations for which transcriptomic profiles are available, preventing the identification of populations in a data-driven and unbiased way. Even when reference profiles are available, immune cells can exhibit tissue-specific expression patterns, a phenomenon that may be even more apparent in tumors. Some cell types are also rare (e.g., mature dendritic cells; ref. 28), or feature very similar gene expression profiles (e.g., subsets of Th cells; ref. 23), which limits our ability to detect them from bulk expression data.

These limitations from bulk approaches can be alleviated using single-cell approaches, such as flow- and mass cytometry as well as single-cell transcriptomics. By profiling many single cells, these approaches enable the application of clustering techniques or other exploratory analyses, such as dimensionality reduction methods, on the collection of cellular profiles itself, enabling the unbiased identification of cell populations from the data. Unlike deconvolution approaches, cytometry transfers the bias from the cell populations assayed to the selection of screened target proteins. Although the number of targets historically has been limited, flow cytometers can now measure a few dozen proteins at single-cell resolution, allowing many exploratory targets to be incorporated into experimental panels. By using heavy-metal tags, mass cytometry can measure the expression of more than 45 distinct protein targets (29). Single-cell transcriptomics allows an even greater number of molecular features to be measured in each cell, at the cost of a reduction in the number of cells that can be profiled (30).

These unbiased approaches not only identify known and expected populations, but also allow the identification of unexpected populations that can arise outside of steady state, such as during infection or cancer (31, 32). In addition, within specific populations, these techniques could reveal an unexpected heterogeneity. For instance, both $CD8^+$ and $CD4^+$ T cells feature tissue-specific expression patterns (33). In the case of cancer, where tumor cells influence the phenotype and functionality of infiltrating cells (34), the ability to measure a high number of distinct cell characteristics, and thus cell phenotypes, appears critical (35, 36). Although the number of proteins quantified by mass cytometry is lower than that of whole-transcriptome analyses, the number of cell phenotypes that can be defined using bimodally expressed markers grows exponentially with dimensionality, allowing a few dozen carefully selected targets to define cellular subsets with high granularity (32).

This high heterogeneity makes summarizing the data at a patient level and thus the identification of features relevant to the disease studied challenging. The development of specific clustering techniques (reviewed and benchmarked in ref. 37) or the application of nonlinear dimensionality reduction techniques (38, 39) has greatly facilitated the exploration and definition of cell subsets from high-dimensional cytometry data. Notably, t-distributed stochastic neighbor embedding (t-SNE), also known as visual t-SNE (viSNE) in the field of cytometry, is a dimensionality reduction technique that allows the projection of data of arbitrarily large finite dimension onto a user-defined number of dimension (typically two), while conserving small-scale structure in the data, so that cells of similar phenotype visually appear clustered together: The algorithm's output focuses on keeping

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points that are close to one another in the high-dimensional space close in the low-dimensional space, effectively representing cells from originating from a given cell population as one or more clusters (38, 39). But the true number of cell subsets present in a collection of samples is nonetheless hard to infer, and overestimating it can lead to a loss of statistical power when trying to correlate the frequency of these subsets to a phenotype of interest, whereas underestimating it may lead to the nonidentification of features relevant to diseases. A needed and active area of research is the development of fully supervised statistical methods, able to define the phenotype of cell subsets that are differentially frequent across groups of samples (40–42). Although these methods are relevant and applicable to any type of immune cell, T cells offer, in addition to the expression of markers, another angle of study through the identification of their cognate antigen.

The Unique Perspective of T Cells

Despite controversy early on, a number of elegant studies show that T cells can effectively control the development of cancer (5). When tumors escape this control, many mechanisms can be involved, including loss of antigen (or ability to present antigen) or the development of immunosuppressive mechanisms. These can lead to T-cell tolerance and dysfunction, such as T-cell "exhaustion" (43) and the expression of various immune checkpoint molecules like PD-1 (14, 44). Hence, cancer immunotherapies, especially checkpoint blockade immunotherapy, likely work by reinvigorating endogenous tumor-specific T-cell responses, leading to the control of tumor growth (45, 46).

In terms of predicting or understanding the bases for effective immunotherapies, major clues should be provided by investigating the tumor-specific T cells themselves: There is good evidence that T cells specific for cancer-specific mutated proteins (neoantigen-specific T cells; ref. 47) can mediate effective tumor regression in both mice (48) and humans (49). Various lines of evidence implicate neoantigen-specific T cells as important contributors to effective checkpoint blockade immunotherapy (6, 48). Altogether, given their role in the disease, it makes sense that the profiles of tumor-specific T cells should be useful in providing biomarkers and insights about how each tumor is evading immune control. However, this comes with serious challenges, mostly surrounding the technical difficulties associated with identifying tumor-specific cells.

Studying immune responses in people is difficult because of experimental limitations and limited access to tissues at the right times. Much progress in understanding tumor immunology has been made by taking advantage of tumor tissues taken as biopsies or during surgical resection (discussed above). However, the most accessible human tissue is blood, and it is fortunate the blood contains large numbers of immune cells, including recirculating T cells of many varieties. Only a small fraction of circulating immune and T cells will be relevant to cancer or other diseases of interest. In the case of T cells and cancer, although the total frequencies of tumor-specific T cells are difficult to assess (due to uncertainties about the range of targets, see below), reports of blood-derived tumor-specific T cells suggest that these frequencies are low (much less than 1% of $CD8^+$ T cells, which typically make up ~10%–20% of peripheral blood mononuclear cells; refs. 50, 51). Therefore, at least in the case of blood, a large part of the signal measured in bulk T-cell profiles should originate from irrelevant cells. In tumor tissues, the problem is certainly less severe, in that tumor-infiltrating T cells are likely enriched for tumor-specific T cells. However, as discussed above, because the immunologic composition of tumor tissues is complex and heterogeneous, similar efforts dedicated to identifying, quantifying, and profiling relevant (tumor-specific) T cells are still needed.

One approach is to use phenotypic markers to identify tumor-specific cells. For instance, sorting of T cells with the highest PD-1 expression enriches for tumor neoantigen–specific T cells from blood (50), providing a good strategy for increasing the signal in downstream analyses. However, in healthy donors, PD-1 is also expressed by relatively higher percentages of nonexhausted memory and effector memory T cells (52, 53). In addition, the expression of PD-1 on tumor-specific T cells has a specific context (melanoma neoantigen–specific cells), and so, it conceivable that tumor-specific T cells may not express increased PD-1 in all other contexts (e.g., perhaps in cases where current immunotherapy approaches are failing). Nonetheless, measuring PD-1 expression represents a major step forward, by focusing attention on T cells in blood that are relevant to cancer.

Another consideration is that although the focus has been on $CD8^+$ T cells, they may be just the tip of the iceberg in terms of antigen-specific tumor responses. Although the participation of $CD4^+$ T cells in tumor control makes sense mechanistically, $CD4^+$ T cells are often ignored due to technical difficulties (the prediction of MHC class II peptide binding is not yet reliable, making it harder to screen). The presence and importance of helper T-cell responses are now being noticed (7, 8, 54, 55). Some B cell–specific responses have also been described (56), but their functional relevance is still poorly understood. In these cases, in the absence of direct identification using peptide–MHC tetramers to identify epitope-specific T cells, measuring TCR or BCR clonality is a way to assess the reactivity of tumor-infiltrating lymphocytes (TILs) toward the corresponding cancer cells.

Making Sense of T-cell Diversity

T cells present highly heterogeneous molecular profiles within and across tissues and conditions. Although the past decades provided major advances in linking molecular markers (cell surface–expressed proteins or transcription factor expression) with functional categories, we cannot yet functionally interpret the diversity of T-cell phenotypes. Studying the phenotype of antigen-specific cells provides a relevant angle to interpret this heterogeneity and leverage it therapeutically (Fig. 1). Human T cells are each endowed with a nearly unique T-cell receptor (57) that leads them each on a unique path of development, followed by a unique context of antigen exposure (for the lucky ones), and results in the generation of an extremely diverse range of phenotypic and functional profiles that can be seen in humans in blood and across tissues (33, 58, 59). Thus, a better understanding of how T-cell profiles are indicative of the context in which they were exposed and are responding to their cognate antigen should provide insights into the overall status of the corresponding immune response. In the case of cancer, the promise is that the features of accurately identified tumor-specific T cells will be informative for the purposes described above. However, several major challenges surround this overall strategy. In general, we need to better understand what the highly diverse profiles of T cells are telling us about status and roles of these cells and perhaps why are they not effectively controlling the tumor. We also need to more easily identify tumor-specific cells, and for this, we are

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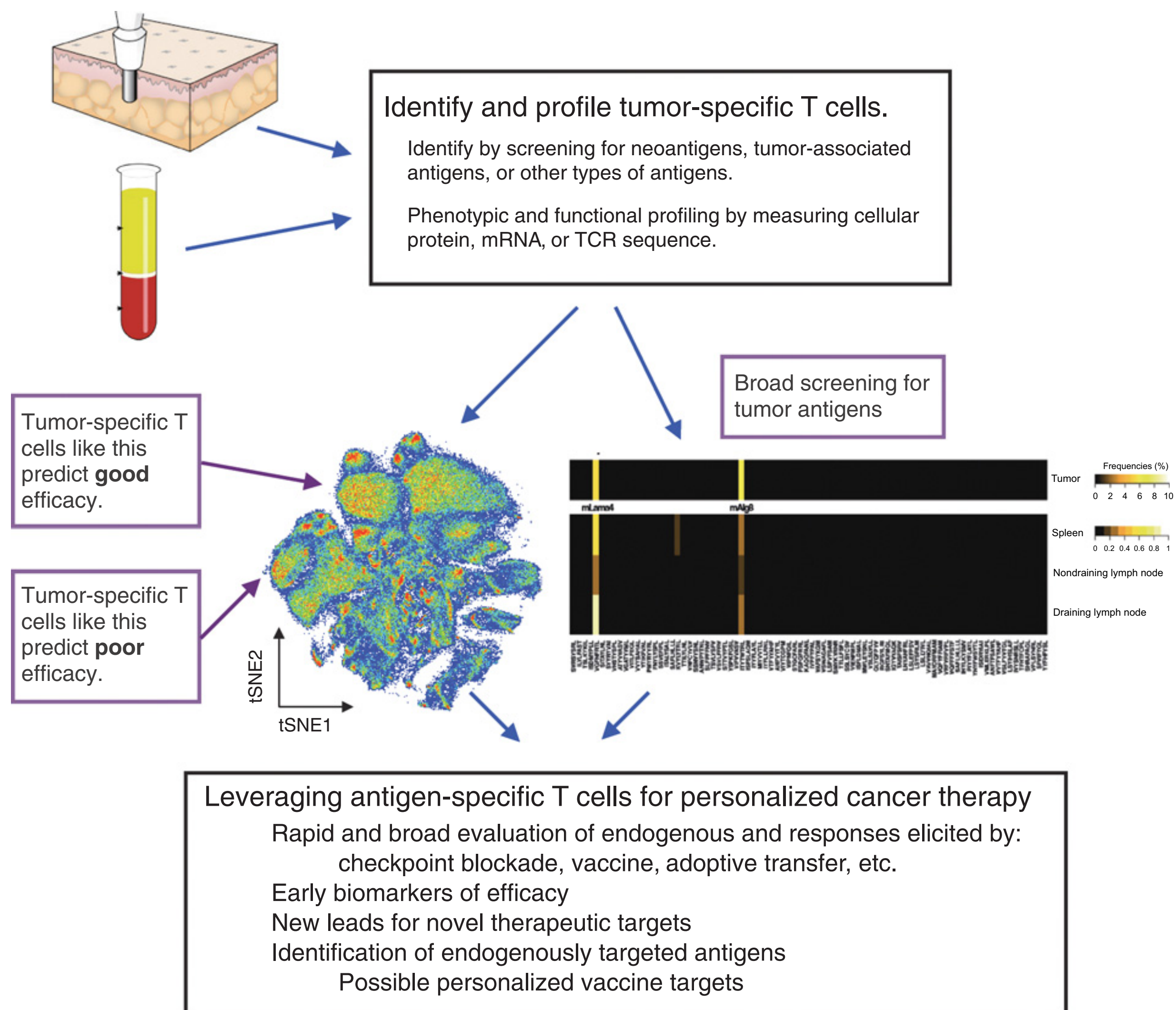

Figure 1.

The potential utility of tumor-specific T cells for cancer-personalized medicine. Although it is currently challenging, patient-derived blood or tumor tissue could be used to routinely screen for, identify, and profile patient-derived tumor-specific T cells. A t-SNE plot is shown, which represents an example of the high diversity of phenotypic profiles observed for human T cells. A better understanding of how the phenotypic profiles of tumor-specific T cells relate to patient outcomes (e.g., responsiveness to checkpoint blockade immunotherapy) should lead to more accurate biomarkers. The identities, antigenic targets, and characteristics of patient-derived tumor-specific T cells could also be used for adoptive cell therapies, vaccine therapies, or as leads for novel therapeutic targets.

challenged by the range of possibilities when it comes to types of antigens that T cells can target. Finally, we need to be practical in knowing that we need to obtain this information from the often-scarce blood and tissue samples that can be collected from patients.

In terms of making sense of the highly diverse profiles of T cells, numerous categories of cellular heterogeneity can be considered. On the basis of our understanding of how T cells work, a logical first step is to segregate T cells broadly into naïve, memory, and effector cell subsets. However, in humans, even these trivial-seeming differentiation states are not simple to directly assess. One important strategy is to identify cellular markers that delineate T cells into different functional subsets. On the basis of this and the observation that CD45 isoforms (CD45RA, CD45RO) and other markers, such as CCR7 and CD27, are variably expressed by human blood–derived T cells, working definitions of naïve, central memory, effector memory, and terminally differentiated effector memory cells have been defined (60). Beyond this, even by probing just a few additional markers, the meaningfulness of each possible subset is difficult to assess, and this issue amplifies exponentially as additional markers are taken into consideration (61). Instead, we can try to ask questions about different types of cellular markers that might be related to either or related to the roles being played by each cell. Defining features of T-cell subsets, especially helper T-cell subsets (e.g., Th1, Th2, Th17, etc.), such as production of various combinations of cytokines, expression of other functionally important molecules, or ability to kill antigen-expressing cells can also be measured for

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individual T cells. We and others have shown that the functional profiles of T cells derived from blood or tissues can be extremely diverse and that neatly grouping T cells into distinct functional subsets may not be feasible when many different parameters are considered (33, 58, 62, 63). Related to their function, T cells can express many different trafficking receptors that influence their localization and movement into and within tissues (64). We tried to tackle this issue by probing a large set of trafficking receptors on all types of lymphocytes isolated from eight different human tissues. Instead of simple rules, we found that coexpression patterns of these receptors are highly diverse and partially overlapping between tissues (33). Nonetheless, an examination of T-cell trafficking receptor profiles may be particularly important in the context of cancer when considering mechanisms of T-cell exclusion from tumors (65). Another important category of markers are "activation" markers, indicative of recent antigen exposure. Activated T cells, expressing markers, such as CD38, HLA-DR, and Ki67, are elevated in blood and peak approximately 1 week after the onset of acute infection, vaccination, or other antigenic challenges. In addition to these roughly defined categories of markers, many other markers can be probed on T cells to assess other important features, such as innate cytotoxic activity (e.g., NKG2D, CD57, and various other NK receptors expressed on differentiated T cells; ref. 66), markers associated with cellular senescence (e.g., CD57 or telomere length), markers associated with exhaustion (e.g., PD-1, Tim-3, Lag-3, TIGIT, etc.; ref. 44), as well as numerous other costimulatory or coinhibitory receptors (e.g., ICOS, 4-1BB, GITR, CD28, CD160, BTLA, CTLA-4, etc.; refs. 53, 67)

In considering the many ways by which T cells can differ from one other and the large number of markers that can be considered for each general category, the possibilities for phenotypic and functional diversity are truly staggering. Now that technologies such as mass cytometry and single-cell sequencing–based approaches are allowing us to assess many of these markers simultaneously, complexity of the coexpression profiles underlying the heterogeneity of T cells is becoming apparent. It may not be so surprising that T cells come in so many different forms, given that their diversity is at least partially driven by the even more staggering diversity derived from the random and inexact TCR gene rearrangements that give rise to each cell's TCR. On this topic, we anticipate that methodologies that broaden our ability to simultaneously assess TCR sequences and the characteristics of the corresponding cells will teach us a great deal about how TCR diversity is related to the extensive phenotypic diversity we observe. To deal with the "curse of dimensionality" that comes from considering many different features of immune cells, we are relying more and more on computer-driven high-dimensional analysis strategies and machine-learning approaches (32, 68) that can help us to identify and understand important features of cellular phenotypes.

Challenges and Approaches for Identifying Tumor-Specific T Cells

Despite the importance of antigen-specific T cells in most adaptive immune responses, it remains extremely difficult to accurately predict epitopes recognized by T cells for any given response. This is because a large number of factors are involved, including, but not limited to, MHC and other genetic factors that influence the range of peptides that can be presented to T cells, the stochasticity of TCR rearrangement and thymic development, and the many layers of complexity involved in antigen processing and presentation (69, 70). In addition, the mechanisms of epitope dominance (i.e., the observation that many T-cell responses are focused on a small number of epitopes) are poorly understood (70). Much effort has been directed at improving algorithms for predicting the capacity of MHC proteins to bind peptides (71, 72). However, these algorithms remain imperfect and only tackle a small piece of the problem when it comes to understanding why any given epitope is or is not targeted by the endogenous T-cell response (73). Thus, much more data are needed to help inform our ability to predict antigen targeted by T cells in humans.

Numerous methods are available for the quantification, identification, and evaluation of antigen-specific T cells. As previously reviewed (74), these methods vary dramatically in terms of their utility and ease of use. Some methods such as ELISpot, intracellular cytokine staining–based assays, proliferation assays, or measurements of antigen-induced changes in cell-surface marker expression have the advantage that they can be easy to perform and do not require precise knowledge of the peptide–MHC epitope. However, these methods make assumptions about the functional abilities of the cells, are affected by baseline T-cell activities, and can be limited in terms of sensitivity and in the extent of phenotypic information obtained. In contrast, methods such as peptide–MHC tetramer or multimer staining allow for the direct identification of antigen-specific cells without perturbation, which may affect measurements of the phenotypic characteristics of the cells, and without the requirement of the functionality of the cells. However, for peptide–MHC multimer-based approaches, knowledge of the exact peptide–MHC epitope is required as well as the ability to produce the recombinant peptide–MHC protein. Advances in the ability to quickly produce both MHC class I and MHC class II multimeric reagents (75, 76), as well as multiplex approaches [based on fluorophores (77, 78), heavy-metal tags (79), or DNA barcodes (80)] that allow for rapid screening, have overcome these limitations to a large degree.

Along these lines, our results demonstrate the utility of a mass cytometry–based highly multiplex peptide–MHC tetramer staining method that allows simultaneous screening of hundreds of different peptide–MHC tetramers combined with an ability to deeply profile the antigen-specific T cells identified at the single-cell level (79). This approach can be used to identify and profile neoantigen-specific T cells in the context of the MCA-sarcoma mouse tumor model (48) of a tumor that is responsive to checkpoint blockade immunotherapy (81). In this study, we used mass cytometry and multiplex tetramer staining to compare the phenotypes of tumor-specific T cells targeting two different tumor antigens derived from tumors versus peripheral tissues from mice treated versus untreated with anti–CTLA-4 or anti–PD-1. From these data, the phenotypic profiles of each cell (tumor specific vs. other cells with unknown antigen specificity) were compared between tissues and treatments. We found that tumor-specific T cell–infiltrating tumors are heterogeneous (even for cells specific for the same epitopes) and that the composition of tumor-specific cells is influenced by the antigen specificity of the cells (i.e., T cells targeting two different neoantigens had consistently different profiles). We also found that checkpoint blockade immunotherapy greatly alters the phenotypes of tumor-specific cells in tumors, without significantly altering the frequencies or phenotypes of

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tumor-specific cells in peripheral tissues in this model (81). Beyond this proof of principle in mice, efforts are ongoing to identify and profile tumor neoantigen-specific T cells from unexpanded human TILs in a similar manner.

Although new approaches based on peptide–MHC multimers are promising, and particularly effective for identifying T cells specific for tumor mutation-derived neoantigens, they still rely on a candidate antigen approach, that is, by screening hundreds or even thousands of antigens, which allows for very comprehensive screening of candidate neoantigens, all possible types of tumor antigens (e.g., oncofetal, oncoviral, overexpressed tissue antigens, cancer–testis, lineage restricted, posttranslationally altered, idiotypic, endogenous retrovirus, etc.) have not been comprehensively screened. Thus, a hypothesis or other insights concerning what T cells are targeting in cancer are still needed. For this reason, we think that multimer-based screening approaches should be complementary with other unbiased approaches for identifying T-cell ligands, such as peptide–MHC yeast display methods that use soluble TCR proteins (cloned from T cells of interest, such as tumor-infiltrating T cells of particular phenotypes) to stain very large libraries ($\sim 10^8$) of yeast each expressing a single peptide–MHC complex (83). Beyond neoantigens and other previously described tumor-associated antigens, it would be very exciting if approaches like this can be used to discover new classes of tumor antigens being targeted by T cells. Once these new classes are discovered, it should be possible to use peptide–MHC multimer–based approaches to screen for, identify, and profile T cells specific for additional related epitopes.

High-throughput sequencing of TCRs also holds great promise for better understanding the human T-cell repertoire and the roles of antigen-specific T cells in cancer (84). For instance, single-cell sequencing of TCRs in parallel with the phenotypic and functional analysis individual cells is particularly powerful (82). One such approach has been used to study the TCR sequence usage of individual $CD4^+$ T cells sorted from colorectal tumor infiltrates, showing that effector-like Th cells and $FoxP3^+$ Treg T cells can express the same TCR clonotype, which implies that these two cellular subsets can be derived from the same T-cell clone (85). However, in this case, the antigen specificity of these cells was not determined. To bridge this gap, articles leveraging knowledge of T-cell antigen specificity to identify sequence motifs associated with particular specificities are exciting (86, 87). However, we caution that these approaches are still far from the possibility of generally predicting antigen specificity based only on TCR sequence. This is because many variables are difficult to account for, such as highly variable T-cell responses in the diversity of clones elicited and because it is not always straightforward to determine the restriction element for any given T cell. In addition to HLA-A, B, C, DR, DP, and DQ, many T cells are restricted by nonclassical MHC ligands, such as HLA-E, HLA-F, various isoforms of CD1, MR1, etc (88). Thus, although thoughtful analysis of TCR sequence analysis based on an understanding of T-cell antigen specificity will undoubtedly be extremely powerful and lead to many new insights, the extent to which T-cell antigen specificity can be inferred based only on TCR sequence alone remains to be seen.

Promises for the Future

With new profound immunotherapy approaches for treating patients being validated at an accelerating pace, it is an exciting time to be an immunologist. With T cells, in particular, in the spotlight for cancer immunotherapy, and based on the many lines of evidence for importance of T cells in controlling cancer, here, we have argued for focusing immune profiling efforts on tumor antigen–specific T cells. Therefore, it also exciting that methods for identifying and analyzing these cells are also rapidly improving. Nonetheless, finding tumor-specific T cells in blood or tissue samples from any given cancer patient remains a difficult task with many avenues open for improvement. We anticipate that major progress will come from better ways to predict T-cell epitopes, through the discovery of additional types of tumor antigens involved in tumor-specific immune responses and through better ways of leveraging TCR sequencing–based approaches. An ability to reliably identify and deeply profile tumor-specific cells from patient samples will greatly improve our ability to understand status of the tumor-specific immune response at personalized level. This should translate into more reliable biomarkers and novel treatments based on antigen-specific T cells and their targets.

Disclosure of Potential Conflicts of Interest

E.W. Newell is an advisor and on the board of directors at Immunoscape Pte. Ltd., has received speakers bureau honoraria from Bristol-Myers Squibb. No potential conflicts of interest were disclosed by the other author.

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